



Europäisches Patentamt
European Patent Office
Office européen des brevets



⑪ Publication number : 0 267 208 B1

⑫

EUROPEAN PATENT SPECIFICATION

④⑤ Date of publication of patent specification :
28.08.91 Bulletin 91/35

⑤① Int. Cl.⁵ : A61K 37/26

②① Application number : 87902450.3

②② Date of filing : 14.04.87

⑧⑥ International application number :
PCT/DK87/00038

⑧⑦ International publication number :
WO 87/06137 22.10.87 Gazette 87/23

⑤④ AN INSULIN PREPARATION FOR NON-PARENTERAL ADMINISTRATION.

③⑩ Priority : 18.04.86 DK 1792/86

④③ Date of publication of application :
18.05.88 Bulletin 88/20

④⑤ Publication of the grant of the patent :
28.08.91 Bulletin 91/35

⑧④ Designated Contracting States :
AT BE CH DE FR GB IT LI LU NL SE

⑤⑥ References cited :
EP-A- 0 128 831
DK-B- 135 268
SE-B- 382 452
US-A- 4 153 689
US-A- 4 534 894
Galenics of Insulin, (1987) Springer Verlag
Heidelberg Berlin, p 20
BIOLOGICAL CHEMISTRY, HOPPE SEYLER,
vol. 366, pp. 521-525, May 1985; Wolfgang H.
Fischer et al.: "A Shortened Insulin with Full in
vitro Potency"

⑤⑥ References cited :
HOPPE SEYLER'S ZEITSCHRIFT FUER
PHYSIOLOGISCHE CHEMIE, Bd. 356, S.
1397-1404, September 1975; Hans-Greger
Gattner: "Darstellung und Eigenschaften
von Des-Pentapeptid (B26-30)-Rinderinsulin"
EUR. J. BIOCHEM. 31, 470-473 (1972); Derek E.
Massey et al: "The Effect of Acylation on the
Molecular Size of Insulin"
BIOCHEMISTRY 1980, 19, 5926-5931; Fredrick
H. Carpenter et al: "Tetrakis(3-nitrotyrosine))
Insulin"

⑦③ Proprietor : NOVO NORDISK A/S
Novo Allé
DK-2880 Bagsvaerd (DK)

⑦② Inventor : BALSCHMIDT, Per
Tibberup Allé 20
DK-3060 Espergaerde (DK)
Inventor : HANSEN, Finn, Banned
Osterbrogade 54 B, 4
DK-2100 Copenhagen (DK)
Inventor : JOHANSEN, Bente, Rose
Garderhojvej 24
DK-2820 Gentofte (DK)

⑦④ Representative : Christensen, Aksel et al
c/o Dansk Patent Kontor A/S H.C. Orsted's Vej
70, Postbox 237
DK-1879 Frederiksberg C (DK)

Note : Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

Description

The present invention relates to insulin preparations for non-parenteral administration.

Ever since the discovery of insulin and its successive use for the treatment of diabetes the only form of administration used in practice has been subcutaneous or intramuscular injection, generally several times a day. In spite of a rapid technical development, this administration still involves a number of disadvantages, such as pain, risk of infection as well as a possibility of chronic tissue injuries. Consequently, there has always been a wish to administer the insulin non-parenterally, and thus in particular oral, rectal, nasal, as well as ophthalmic administrations have been investigated.

The ophthalmic administration of insulin has been disclosed in DK-A-135268, according to which specification an insulin-containing hydrogel is placed under the eyelid. Thereby an absorption of 5-20% of the amount of insulin administered is obtained over a period of several hours.

The oral administration demands that special protective measures are taken in order to prevent the insulin from being decomposed, before absorption can take place through the intestinal mucosa. This can e.g. be done as stated in DK-A-1925/83 and 2294/84, according to which enzyme inhibitors are added and an encapsulation in a bile-soluble material may be made. Oral administration only gives an absorption of 1-2% of the amount of insulin administered, cf. Galloway et al., *Diabetes* 21 (1971), pages 640-648.

Rectal insulin administration is disclosed in e.g. DE-A-2641819, US-A-4164573 and 4442090. In this way of administration the insulin is compounded in the form of a suppository in which the presence of an enzyme inhibitor is normally not necessary. Hereby an absorption of the amount of insulin administered of about 5% can be obtained.

In the forms of administration mentioned above use is made in almost all cases of a surface-active additive to increase the absorption rate and efficiency of the insulin through the mucosae. An example of such absorption promoting adjuvants are polyoxy-ethylene fatty alcohol ethers and in particular bile acids or salts thereof.

Such additives are particularly effective in nasal administration of insulin, vide FR-A-2321275 and GB-A-1554157, and the technique has been further described by S. Hirai et al., *Diabetes* 27 (1978), pages 296-299, and G.S. Gordon et al., *PNAS* 82 (1985), pages 7419-7423. In this form of administration the insulin is administered in solution as drops or better in atomized form to the nasal cavity, whereafter the absorption through the nasal mucosa occurs so rapidly that the maximum insulin concentration is obtained already after 10 to 30 minutes. This rapidly commencing absorption is of particular therapeutical importance as the maximum insulin concentration in normal subcutaneous injection of dissolved insulin does not commence until after 30-60 minutes, which may cause problems in a much employed diabetes therapy, in which injections of rapidly acting insulin are given immediately before the principal meals.

However, in the nasal form of administration an adverse effect on the mucosae due to the added absorption promoting adjuvants has been demonstrated after some time, vide S. Hirai et al., *Int. J. Pharm.* 9 (1981), pages 173-184.

This problem is said to be solved by the use of certain fusidic acid or cephalosporine derivatives as adjuvants, vide EP-A-128831. The proportion of insulin absorbed by nasal administration in dissolved and atomized form is said to be up to about 20% in the publications mentioned above.

An embodiment of the nasal administration in which the use of surface-active adjuvants can be avoided is known from EP-A-122036. Said embodiment consists in the administration of the insulin as a powder, whereby use is made of a water-insoluble, but water-absorbing carrier, e.g. crystalline cellulose. Hereby the particles adhere to the nasal mucosa, and moisture is absorbed which has the effect that the insulin attains an intense contact with the mucosa and thereby can be absorbed with a high efficacy.

In all the known insulin preparations for non-parenteral administration a conventional commercially available insulin is used as the basis of the formulation. When such an insulin is dissolved at physiological pH value a concentration-dependent equilibrium is established between monomeric, dimeric, hexameric, and polymeric insulin. The degree of self-association can be determined e.g. by ultracentrifugation or by gel filtration methods, vide e.g. R. Valdes Jr. and G.A. Ackers, "Methods in Enzymology", Vol. 61 (Enzyme Structure, part H, ed.: Hirs & Timasheff), Academic Press 1979, pages 125-142. In conventional commercially available preparations of dissolved insulin a very large proportion of the insulin is present as hexamers.

By the use of insulin derivatives which in solution in the physiological pH range predominantly are present as monomers in insulin preparations for non-parenteral administration a still more rapid absorption of the insulin activity administered is obtained, as maximum concentration in serum is attained already after 5-10 minutes.

The insulin preparations of this invention are characterized in that they contain one or more insulin derivatives which in solution in the physiological pH range predominantly are present as monomers, usual additives and one or more absorption promoting substances. The physiological pH range comprises pH values from about 6.5 to about 8.5.

In particular, the invention relates to insulin preparations for nasal administration.

Nasal insulin preparations may e.g. be dosed from a bottle equipped with a manual atomizer designed for delivering a specific volume of insulin solution per dose. Thus, the nozzle of the bottle is led into a nostril before the atomization which may be repeated in the other nostril.

Insulin derivatives which predominantly are present as monomers often exhibit reduced insulin activity. Thus, such insulin derivatives that still exhibit a considerable insulin activity, e.g. A1, B1, B29-trisuccinyl insulin, tetrakis-(3-nitrotyrosine)-insulin as well as des-pentapeptide(B26-30)-insulin, des-pentapeptide(B26-30)-insulin-B25-amide, and sulphated or phosphorylated insulin, are useful in the preparation of the invention.

Trisuccinyl insulin can be prepared by treating insulin with succinic acid anhydride (D.E. Massey, D.G. Smyth : Eur. J. Biochem. 31 (1972), pages (470-473); tetranitro-insulin can be obtained by reaction between insulin and tetranitro methane (F.H. Carpenter et al., Biochemistry (USA) 19 (1980), pages 5926-5931); des-pentapeptide-insulin is formed by digesting insulin with pepsin (H.G. Gattner : Hoppe-Seyler's Z. Physiol. Chem. 356 (1975), pages 1397-1404); des-pentapeptide-insulin amide can be prepared by condensing des-octapeptide insulin and gly-phe-phe-NH₂ by trypsin-assisted peptide bond formation (W.H. Fischer et al., Biol. Chem. Hoppe-Seyler 366 (1985), pages 521-525), and sulphated insulin may be obtained by reacting insulin with a carbodiimide in the presence of sulphuric acid (S. Pongor et al., Diabetes 32 (1983), pages 1087-1091, US-A-4534894).

Useful absorption promoting substances are surface-active compounds, as e.g. fatty alkyl or fatty acyl substituted polyethyleneglycol, block copolymers of polyethyleneglycol and polypropyleneglycol, salts of bile acids, as e.g. taurocholic acid, deoxycholic acid and glycodeoxycholic acid, salts of N-acyl amino acids, as e.g. lauroyl sarcosine and palmitoyl glutamic acid, as well as derivatives of fusidic acid and cephalosporine. Particularly useful are such substances exhibiting the least possible tissue irritation.

Physiologically acceptable acids or bases of pH values near the physiological pH range can be used as buffers, e.g. phosphates, tris(hydroxymethyl)-aminomethane (TRIS) and glycyl glycine. Physiologically acceptable salts or polyhydroxy compounds can be used as isotonic, e.g. sodium chloride, glycerine or mannitol. Useful preserving agents comprise phenol or benzyl alcohol or similar substances being active in the physiological pH range.

Useful suppository vehicles in preparations for rectal administration are premixed synthetic fats, e.g. "Witepsol"®, or partly decomposed vegetable oils and fats or Macrogols.

In powderous preparations for nasal administration dried water-absorbing, but water-insoluble substances, e.g. cross-linked polyvinyl pyrrolidone or in particular crystalline cellulose having a particle size between 20 and 200 µm can be used as carriers.

Aqueous preparations intended for nasal administration generally contain 40-500 I.U. insulin activity per ml, preferably ranging from 80 to 300 I.U./ml. The content of surface-active absorption promoting substances is usually in the range of from 0.1 to 10%, and a content of from 0.5 to 5% is preferred. The pH of the preparation should be near the physiological pH and a pH range between 7 and 8 is preferred.

Powders for nasal administration generally contain 0.5-10 I.U. insulin activity per mg, preferably 1-8 I.U./mg. The insulin or insulin derivative used in these preparations should be prepared as an easily water-soluble product, preferably by lyophilization from solutions having a pH of between 6.5 and 8.5.

Rectal insulin preparations in the form of suppositories generally contain 2-200 I.U. insulin activity per gram, of which 5-100 I.U./gram is preferred. The absorption promoting substance is usually added in an amount of 0.1-25% by weight, and 0.5-10% (w/w) is preferred. The preparations are generally cast as suppositories of 0.5-3 grams, preferably weighing 1-2.5 grams.

The invention is further illustrated by the following Examples.

EXAMPLE I

Formulation of a preparation for rectal administration.

95 mg of trisuccinyl porcine insulin and 1.0 gram of sodium cholate were thoroughly mixed into 100 ml of melted "Witepsol"® at 36°C, and the resulting mass was cast in forms to suppositories of 2 ml, each containing 20 I.U. of insulin activity.

EXAMPLE II

Formulation of a preparation for nasal administration.

75 mg of des-pentapeptide(B26-30) porcine insulin-B25-amide were dissolved in 3 ml of water by means

of a small amount of hydrochloric acid, whereafter 5 ml of 0.02 M sodium dihydrogen phosphate in 1% phenol were added. The pH value was adjusted to 8.5 with sodium hydroxide, and water was added until 10 ml. This solution was now mixed with 10 ml of a freshly produced 2% solution of sodium glycodeoxycholate in 0.25 M sodium chloride, adjusted to a pH value of 7.5 with hydrochloric acid and subsequently filled into a bottle, which
 5 was sealed with a manual atomizer delivering a specific volume of solution per puff. With a dosage volume of 100 µlitres 10 I.U. of insulin activity was thus administered through a single puff.

EXAMPLE III

10 Preparation of des-pentapeptide(B26-30) porcine insulin-B25-amide.

500 mg of des-octapeptide(B23-30) porcine insulin prepared in a manner known per se by trypsin digestion of porcine insulin and 1000 mg of gly-phe-phe-NH₂ prepared in a manner known per se by Merrifield-synthesis were dissolved in a mixture of 6 ml of dimethylformamide and 3 ml of water, and the pH value was adjusted to
 15 6.5 with triethylamine or acetic acid. A solution of 25 mg trypsin in 500 µlitres of 0.001 M calcium acetate was added with stirring, and the reaction mixture was left at 20°C for 4 hours. Then the proteins were precipitated from the solution by the addition of 100 ml of acetone and separated by centrifugation. The precipitate was redissolved in 20 ml of 1 M acetic acid, 7 M urea and gel-filtered on a 5 × 85 cm column of "Sephadex"® G 50 Superfine in 1 M acetic acid. The fractions containing the protein main fraction were collected and freeze-
 20 dried.

The freeze-dried protein was redissolved in 40 ml of 7 M urea, the pH value being adjusted to 8.1 with 1 M TRIS and applied to a 2.6 × 20 cm column of DEAE-cellulose ("Whatman"® DE-52), packed and equilibrated in 0.02 M TRIS/hydrochloric acid, 7 M urea pH value 8.1. Thereafter the column was eluted at 4°C with a sodium chloride gradient from 0.1 M in the same buffer over 48 hours at a flow of 40 ml per hour. The fractions containing
 25 the protein main peak first eluted were collected and desalted on "Sephadex"® G 25 Fine in 1 M acetic acid. The proteinaceous pool was freeze-dried.

Yield : 260 mg of des-pentapeptide(B26-30) porcine insulin-B25-amide.

EXAMPLE IV

30 Preparation of trisuccinyl human insulin.

250 mg of Zn-free semisynthetically prepared human insulin were dissolved in 12.5 ml of 0.5 M TRIS/acetic acid, 7 M urea pH 8.5, whereafter 25 mg of succinic anhydride were added with stirring. The mixture was left
 35 with stirring for 1 hour at 20°C. Thereafter the reaction mixture was desalted on "Sephadex"® G 25 in 0.05 M ammonium hydrogen carbonate, and the proteinaceous fractions were freeze-dried. The freeze-dried substance was dissolved in 1.5 M acetic acid, 0.05 M sodium chloride, 7 M urea and applied to a 2.6 × 20 cm column of SP-"Sephadex"® C-25, packed and equilibrated in the same buffer. Then there was eluted with a 0.05 →
 40 0.2 M sodium chloride gradient over 24 hours at a flow of 40 ml per hour at 4°C. The protein main peak first eluted was collected and desalted on "Sephadex"® G 25 in 0.05 M ammonium hydrogen carbonate. The proteinaceous eluate was freeze-dried.

Yield : 125 mg of trisuccinyl human insulin.

EXAMPLE V

45 Preparation of sulphated porcine insulin.

250 µlitres of concentrated sulphuric acid were added dropwise with stirring to 4.75 ml of ice-cold anhydrous dimethyl formamide, whereupon 100 mg of Zn-free porcine insulin were dissolved therein with continued stir-
 50 ring. 500 mg of dicyclohexylcarbodiimide dissolved in 1 ml of dimethyl formamide were added, and the mixture was shaken at 4°C for 6 hours. Hereafter about 5 grams of crushed ice were added, and the mixture was neutralized with 10 N sodium hydroxide solution. 10 ml of water were added, and the precipitated dicyclohexyl urea was separated by centrifugation. Thereafter the supernatant was desalted on a "Sephadex"® G 25 column in 0.05 M ammonium hydrogen carbonate solution and freeze-dried.

55 Yield : 80 mg of sulphated porcine insulin.

EXAMPLE VI

Formulation of a preparation for nasal administration.

- 5 70 mg of sulphated porcine insulin were dissolved with stirring in 10 ml of 0.14 M of sodium chloride containing 1% of sodium-tauro-24,25-dihydro-fusidate and 0.3% m-cresol, the pH value being adjusted to 7.4. Hereby a preparation containing 150 I.U. of insulin activity per ml is obtained, which preparation is particularly suitable for administration from a spray dosage container due to the good shaking stability of the insulin derivative

10

EXAMPLE VII

Formulation of a preparation for nasal administration.

- 15 100 mg of des-pentapeptide (B26-30) porcine insulin-B25-amide were dissolved in 20 ml of water with the pH value being adjusted to 7.5 with sodium hydroxide, and the solution was freeze-dried. The resulting insulin powder was thoroughly mixed in a mortar with 800 mg of crystalline cellulose so as to obtain a uniform powder composition containing 3 I.U. of insulin activity per mg.

- 20 Thereafter the nasal powder can be administered to the nasal mucosa by means of an insufflator, which may be designed in such a way that the powder is administered as a dosed medicine in the form of a gelatine capsule being perforated immediately before the insufflation.

EXAMPLE VIII

- 25 In vivo experiments.

- Male, 6 weeks old, Wistar rats ($150 \pm$ grams) made diabetic 3 days prior by intravenous injection of streptozotocin (65 mg/kg) were anaesthetized with pentobarbital (50 mg/Kg) intraperitoneally, and polythene catheters were inserted into a carotid artery for blood sampling. A tracheal cannula was inserted in the rats before the intranasal insulin administration.

30

Groups of 4 rats were dosed intranasally at 0 min. with 10 μ litres of one of the following preparations :

- A. 250 I.U./ml of Zn-insulin (human)
 1/75 M sodium phosphate
35 1.6% (vol/vol) of glycerol
 1.0% (weight/vol) of sodium glycodeoxycholate
- B. 250 I.U./ml of des-pentapeptide (B26-30) porcine insulin-B25-amide
 1/75 M sodium phosphate
40 1.6% (vol/vol) of glycerol
 1.0% (weight/vol) of sodium glycodeoxycholate
- C. 1/75 M sodium phosphate
 1.6% (vol/vol) of glycerol
45 1.0% (weight/vol) of sodium glycodeoxycholate

250 μ litres of blood were sampled into heparinized ice-cold tubes at -5, 10, 20, 40, 60, 90, and 120 minutes, and the plasma samples were frozen after centrifugation for later insulin determination by radioimmunoassay.

- 50 Table I and Fig. 1 depict the plasma insulin levels (means and S.E.M.) at the various sampling times. The detection limit is 0.05 nmol/litre.

It can be seen that the monomeric des-pentapeptide insulin-amide (B) was absorbed faster and more reproducibly than the conventionally used hexameric insulin (A).

55

TABLE I

Preparation	No. of rats		Plasma insulin concentration (nmol/litre)						
			minutes after administration						
			-5	10	20	40	60	90	120
A		\bar{x}	<0.05	18.07	18.14	16.32	5.97	3.21	1.21
(Zn-insulin)	4	\pm S.E.M.:		± 6.81	± 6.87	± 6.42	± 2.17	± 0.99	± 0.34
B		\bar{x}	<0.10	59.39	19.46	7.13	3.88	1.42	0.96
(DPI-amide)	4	\pm S.E.M.:		± 14.25	± 2.74	± 1.83	± 1.17	± 0.46	± 0.26
C		\bar{x}	<0.05	<0.08	<0.07	<0.06	<0.15	<0.08	<0.09
(placebo)	4	\pm S.E.M.:							

Claims

1. Insulin preparation for non-parenteral administration, characterized by containing one or more insulin derivatives, which in solution in the physiological pH range predominantly are present as monomers, usual additives and one or more absorption promoting substances.
2. Insulin preparation according to claim 1, characterized by being a preparation for nasal administration.
3. Insulin preparation according to claims 1 or 2, characterized in that the insulin derivative is des[(B26-30)-pentapeptide]insulin-B25-amide.
4. Insulin preparation according to claims 1 or 2, characterized in that the insulin derivative is des[(B26-30)-pentapeptide]insulin.
5. Insulin preparation according to claims 1 or 2, characterized in that the insulin derivative is A1, B1, B29-trisuccinyl insulin.
6. Insulin preparation according to claims 1 or 2, characterized in that the insulin derivative is sulphated insulin.
7. Use of insulin derivatives which in solution in the physiological pH range predominantly are present as monomers for the formulation of insulin preparations for non-parenteral administration.

Patentansprüche

1. Insulinpräparation zur nichtparenteralen Verabreichung, dadurch gekennzeichnet, daß sie ein oder mehrere Insulinderivate, die in Lösung im physiologischen pH-Bereich überwiegend als Monomere vorliegen, sowie übliche Additive und eine oder mehrere absorptionsfördernde Substanzen enthält.
2. Insulinpräparation nach Anspruch 1, dadurch gekennzeichnet, daß sie eine Präparation zur nasalen Verabreichung darstellt.
3. Insulinpräparation nach anspruch 1 oder 2, dadurch gekennzeichnet, daß das Insulinderivat Des[(B26-30)-pentapeptid]-insulin-B25-amid ist.
4. Insulinpräparation nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß das Insulinderivat Des [(B26-30)-penpapeptid]-insulin ist.
5. Insulinpräparation nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß das Insulinderivat A1, B1, B29-Trisuccinylinsulin ist.
6. Insulinpräparation nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß das Insulinderivat sulfatiertes Insulin ist.
7. Verwendung von Insulinderivaten, die in Lösung im physiologischen pH-Bereich überwiegend als Monomere vorliegen, zur Formulierung von Insulinpräparationen zur nichtparenteralen Verabreichung.

Revendications

1. Préparation d'insuline pour l'administration non parentérale, caractérisée en ce qu'elle contient un ou plusieurs dérivés d'insuline qui en solution dans la gamme de pH physiologique sont présents principalement sous forme de monomères, des additifs habituels et une ou plusieurs substances favorisant l'absorption.
2. Préparation d'insuline selon la revendication 1, caractérisée en ce que c'est une préparation pour l'administration par voie nasale.
3. Préparation d'insuline selon la revendication 1 ou 2, caractérisée en ce que le dérivé d'insuline est le dé-[pentapeptide (B26-30)]insuline-B25-amide.
4. Préparation d'insuline selon la revendication 1 ou 2, caractérisée en ce que le dérivé d'insuline est la dé-[pentapeptide (B26-30)]insuline.
5. Préparation d'insuline selon la revendication 1 ou 2, caractérisée en ce que le dérivé d'insuline est la A1, B1, B29-trisuccinyl-insuline.
6. Préparation d'insuline selon la revendication 1 ou 2, caractérisée en ce que le dérivé d'insuline est l'insuline sulfatée.
7. Utilisation de dérivés d'insuline qui en solution dans la gamme de pH physiologique sont principalement présents sous forme de monomères pour la formulation de préparations d'insuline pour l'administration non parentérale.

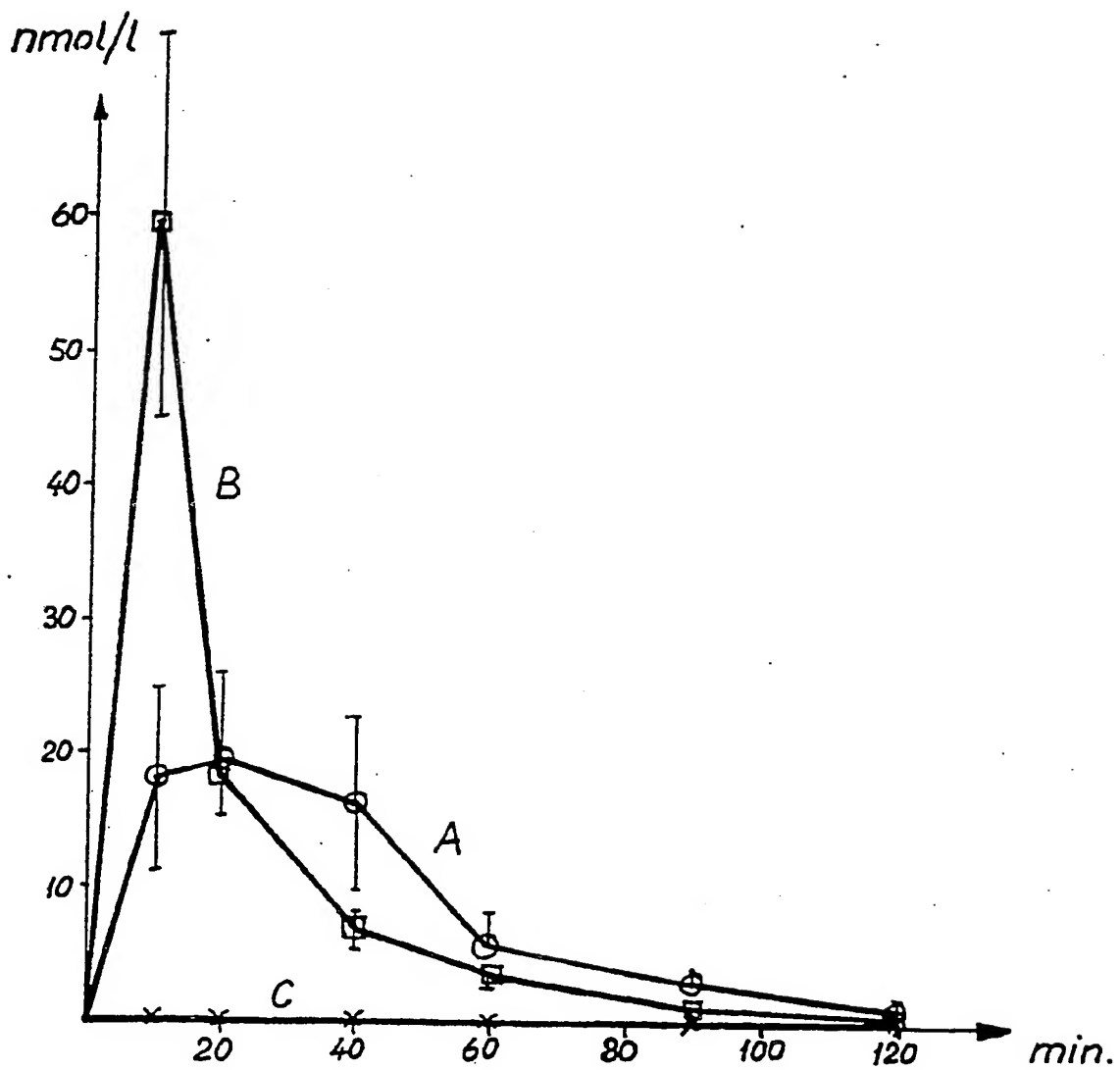


Fig. 1

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☒ **FADED TEXT OR DRAWING**
- ☒ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☒ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.